

## Influence of autochthonous lactic acid bacteria on the proteolysis, microstructure and sensory properties of low fat UF cheeses during ripening

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Received - Prispjelo: 26.03.2012.

Accepted - Prihvaćeno: 24.05.2012.

### Summary

The influence of commercial bacteria *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* (cheese A) and combinations of autochthonous lactic acid bacteria (LAB) strains *Lactobacillus paracasei* ssp. *paracasei* 08, *Lactococcus lactis* ssp. *cremoris* 656, *Lactococcus lactis* ssp. *lactis* 653 (cheese B and C) on composition, proteolysis, microstructure and sensory properties of low fat cheeses during ripening was investigated. Low fat cast ultra-filtered (UF) cheeses were produced according to the defined production procedure by mixing UF milk protein powder, skim milk and cream. Significant influence of different LAB strains on composition, primary proteolysis and microstructure was not found. Cheeses made with autochthonous LAB showed a higher rate of secondary proteolysis, as well as higher flavour scores, and were more acceptable than control cheese.

*Key words:* cheese, starter culture, microstructure, sensory properties

### Introduction

In the past 20 years the popularity and commercialization of food that could be beneficial for human health significantly increased around the world. Dairy products, including cheeses, especially those with reduced fat content, represent a good base for development of new products with functional properties.

White brined cheeses are the most widely produced and consumed cheeses in Serbia (about 60 % of total cheese consumption) (Radulovic et al., 2011) as well as in the Mediterranean region (Alichanidis and Polychroniadou, 2008). However, over the last couple of decades an alternative, the application of ultrafiltration process has become the most common way of white cheeses production.

The most commonly produced cheese using ultrafiltration process is known as cast UF cheese and is usually produced as full fat cheese. The manufacture of cheese from recombined milk is now a well established technology for making a wide variety of cheeses, especially fresh cheeses such as Domiati, Feta and Cottage cheese (Jana and Thakar, 1996). Utilisation of UF milk protein concentrate for production of brined cheeses represents an alternative procedure to the UF process *in situ* and offers an economic possibility for the manufacture of UF cheese. It is particularly important for developing countries as well as countries with low milk production (Jana and Thakar, 1996). Selection of proper milk protein powder and optimal homogenization are two the most critical factors influencing cheese quality (Jana and Thakar, 1996).

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Low fat cheeses generally refer to cheeses which fat content is lower than its corresponding full fat variety. It is known that fat, apart from its nutritional significance in cheese, also contributes to sensory and functional properties of dairy products. Due to the reduction of fat in cheese, there is a shift in the balance of the various components compared to its full fat counterpart. Hence, cheeses with reduced fat content, especially hard varieties, are usually characterized as having atypical and/or less acceptable sensory properties, especially textural and rheological (Banks, 2004). Several approaches have been investigated as potential ways to improve the flavour and texture of low-fat cheeses, e.g. modification of conventional production procedure, additives (fat replacers), specially designed starter cultures or adjunct cultures, use of enzymes such as plant proteases etc. (Fenelon et al., 2002).

The addition of adjunct cultures has been quite promising in low-fat cheese manufacture and resulted in enhancement of cheese flavour. Numerous research studies were performed worldwide in order to investigate the possibility of different cheeses with reduced fat content production by different starter cultures addition in order to achieve acceptable sensory properties i.e. Cheddar (Fenelon et al., 2002; Guinee et al., 2000), Feta (Katsiari et al., 2002b; Michaelidou et al., 2003a), Edam (Tungjaroenchai et al., 2001), Kefalograviera (Katsiari et al., 2002a; Michaelidou et al., 2003b). Several authors reported great potential of lactic acid bacteria (LAB) isolated from traditionally made cheeses that can be used as starter or adjunct cultures for standardization or improvement of sensory properties of different cheese types, including low fat ones (El Soda et al., 2007; Radulovic, 2007). Improvement of cheese sensory properties, especially of aroma, by using adjunct cultures, has mainly been associated with enhancement of proteolysis and with increased concentration of small peptides, particularly of free amino acids in the final cheese (Law, 2010).

The present study is based on the previous research (Miocinovic et al., 2011) which was aimed to define the manufacture of low fat UF cheese. Cheeses were produced from recombined UF milk obtained by mixing UF milk protein concentrate, skim milk powder and cream. The objective of the present study was to determine the effect of

starter culture containing autochthonous strains of LAB, isolated from traditional Serbian white brined cheese (Radulovic, 2007), on the composition, microstructure, proteolysis and sensory properties of low fat UF cheeses during 8 weeks of ripening. Properties of two experimental cheeses produced in this study with autochthonous LAB as a adjunct, or as whole starter culture, were compared to the characteristics of control cheese produced just with commercial LAB and previously presented in study (Miocinovic et al., 2011).

## Material and methods

### Materials

The formulation and production of low fat UF cheeses were carried out with the following raw materials: milk protein isolate Promilk 852 (Ingredia, France), as source of protein component, skim milk powder (Dairy plant Subotica, Subotica, Serbia), as source of lactose component, cream (Polimark, Belgrade, Serbia), as a source of lipids component in UF cheese milk. Water was used as solvent for the hydration of milk protein isolate and skim milk powder, as well as for the final dry matter standardization of UF milk.

### Bacterial cultures

The autochthonous LAB strains included *Lactococcus lactis* ssp. *cremoris* 656, *Lactococcus lactis* ssp. *lactis* 653 and *Lactobacillus paracasei* 08 (Culture Collection of the Department for Food Microbiology, University of Belgrade) and were previously isolated from traditional Serbian brined cheeses (Radulovic, 2007). These strains were selected according to their characteristics important from the biochemical and technological viewpoint, as described by Martinovic et al. (2005). Lactococci strains were cultivated in M17 broth (Merck, Darmstadt, Germany) and lactobacilli in MRS broth (Merck, Darmstadt, Germany) for two transfers at 30 °C for 24 h (1 mL inoculum 100 mL<sup>-1</sup>). For propagation, strains were grown separately in sterile reconstituted skim milk (1 mL inoculum 100 mL<sup>-1</sup>, heated at 90 °C for 30 min), as described by Fenelon and Guinee (2000). Cell counts were  $\approx 1 \times 10^9$  cfu mL<sup>-1</sup>.

## Cheese manufacture

In order to obtain the desired cheese composition, UF milk protein powder, skim milk powder and cream were mixed in ratio of 18:17:7.5. Dissolution, hydration and intensive mixing of milk powders was performed at 50 °C during 1 h, followed by heating the concentrate at 85 °C (5 min) and cooling to inoculation temperature of 35 °C. Inulin 1.5 g kg<sup>-1</sup> (Cosucra, Belgium) was added before inoculation of UF milk.

The UF milk was divided into three equal parts and starter cultures were used as follows:

- Cheese A was produced with commercial starter cultures consisting of *Lactococcus lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris* (LL50A, DSM, Netherland) (Miocinovic et al., 2011) which were added directly into milk according to the producer's manual;
- Cheese B was produced with commercial starter cultures and adjunct autochthonous strain *Lb. paracasei* 08 at the level of 4 g kg<sup>-1</sup>;
- Cheese C was produced with an autochthonous starter culture consisting of *L. lactis* ssp. *cremoris* 656, *L. lactis* ssp. *lactis* 653 and *Lb. paracasei* 08, in the ratio of 5:3:2, at the level of 20 g kg<sup>-1</sup> (Radulovic, 2007).

Coagulation was performed by adequate amount of rennet addition (Fromase®, DSM, Netherlands). The UF milk was poured into packaging units of 0.5 kg, where coagulation and fermentation took place at 29-30 °C during 17-18 h. Cheeses were dry salted with the addition of 20 g kg<sup>-1</sup> salt mixture consisting of NaCl and KCl in ratio 3:1 (Kristal So, Belgrade, Serbia). Cheese ripening took place at 12 °C and 5 °C during 56 days. Cheeses were made in three replications.

## Analytical methods

Sampling, determination of composition, proteolysis parameters, electrophoresis and sensory properties of low fat UF cheeses were performed after fermentation (day 0), after 7, 21, 35 and 56 days of ripening. Microstructure of low fat UF cheeses was analysed after 56 days of ripening.

## Cheese composition

Grated cheese samples were analyzed in duplicate for dry matter (DM) by oven drying at 102±2 °C

(IDF, 1982), fat (MF) by Van Gulik method (IDF, 1986), total protein (TP) by Kjeldahl method (IDF, 2002) with Kjeltec System (Tecator 1002, Sweden). The pH of cheese slurry was measured with pH meter (Consort, Belgium).

## Assessment of proteolysis

Cheeses were analyzed for water-soluble nitrogen (WSN) according to the method of Kuchroo and Fox (1982), expressed as a percentage of total nitrogen (WSN/TN). Phosphotungstic acid (5 mL L<sup>-1</sup>) soluble nitrogen (PTAN) of cheeses were analyzed according to Stadhouers method (1960), expressed as a percentage of total nitrogen (PTAN/TN) and of water soluble nitrogen (PTAN/WSN).

*Urea polyacrylamide gel electrophoresis (Urea PAGE)*. Urea PAGE was performed according to Andrews (Andrews, 1983) using a vertical slab unit TV200YK (Consort, Belgium) with 100x200x1 mm slabs, Tris-glycine electrode buffer, constant current of 60 mA and maximum voltage of 300V for 3 h, with 4 % stacking gel (pH 7.6), and 12 % separating gel (pH 8.9).

*Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE)* was performed according to Laemmli (1970) with the same equipment as Urea PAGE, Tris-glycine electrode buffer, using stacking gel of 4 % (pH 6.8) and a 15 % separating gel (pH 8.9), constant current of 80 mA, maximum voltage of 300V for 4 h.

Detected polypeptides were identified using standards of  $\alpha$ - and  $\beta$ -casein (Sigma, USA) and the low molecular weight kit (LKB-Pharmacia, Sweden) consisted of phosphorilase B (94000), bovine serum albumin-BSA (67000), ovalbumin (43000), Carbonic anhydrase (30000), Trypsin inhibitor (20100) and  $\alpha$ -lactalbumin (14400).

The gels were stained with a staining solution (0.23 % Coomassie Brilliant blue R-250, 3.9 % trichloroacetic acid, 17 % methanol, 6 % acetic acid) for 1.5 h and de-stained in de-staining solution (8 % acetic acid, 18 % ethanol). The gel images were recorded using a scanner Bear Paw 2448TA+ (Muster, Germany).

## Scanning electron microscopy - SEM

Cheese samples were cut and prepared according to Kuo and Gunasekaran (2009) and immedi-

ately critical point dried with liquid carbon dioxide using a CPD 030 critical point dryer (BAL-TEC, Scan, Germany). The dried, fractured samples were coated with gold in a sputter coater (SCD 005, BAL-TEC, Germany). The samples were examined in a JEOL JSM-6390 LV scanning electron microscope operated at an accelerating voltage of 13 kV and 1 000x, 5 000x, 10 000x and 20 000x magnification.

### Sensory analysis

The sensory evaluation of chesses was performed by a group of six experts after 7, 21, 35 and 56 days of ripening. Evaluated characteristics were exterior and interior, appearance, body and texture, and flavour (odour and taste) using a 5-point scale, with 1 being the worst and 5 the best quality (Radovanovic and Popov-Raljic, 2001). Depending on the importance of attributes, given points were cor-

rected and multiplied by 1, 4, 10 and 5, respectively. The sum of corrected scores gave the "percentage of total sensory quality" which maximum is 100 (Joksimovic, 1977).

### Statistical analysis

Data were analysed using STATISTICA 6.0 (StatSoft, USA) data analysis software. LSD test was used to determine differences among cheeses at a 0.05 statistical level.

## Results and discussion

### Cheese composition and pH values

The composition of the experimental cheeses during ripening is summarized in Table 1.

There were no significant differences ( $P>0.05$ ) in the gross composition of control and experimental low fat UF cheeses. However, cheeses made with

Table 1. Composition and pH of low fat UF cheese during ripening

Days	Cheese*	Dry matter (%)	Protein (%)	Moisture in nonfat part (%)	Fat in dry matter (%)	pH
0	A	24.69±0.42 <sup>a</sup>	15.94±0.33 <sup>a</sup>	78.31±0.61 <sup>a</sup>	15.54±1.19 <sup>a</sup>	4.85±0.03 <sup>a</sup>
	B	24.87±0.55 <sup>a</sup>	16.46±0.74 <sup>a</sup>	78.26±0.57 <sup>a</sup>	15.43±1.14 <sup>a</sup>	4.84±0.03 <sup>a</sup>
	C	23.70±0.32 <sup>b</sup>	15.52±0.34 <sup>b</sup>	79.34±0.50 <sup>a</sup>	16.18±1.34 <sup>a</sup>	4.98±0.07 <sup>b</sup>
7	A	27.03±0.59 <sup>a</sup>	16.44±0.75 <sup>a</sup>	75.88±0.75 <sup>a</sup>	14.20±1.10 <sup>a</sup>	4.71±0.05 <sup>a</sup>
	B	27.23±0.53 <sup>a</sup>	16.90±0.64 <sup>a</sup>	75.67±0.58 <sup>a</sup>	14.08±0.92 <sup>a</sup>	4.61±0.06 <sup>a</sup>
	C	26.01±0.78 <sup>b</sup>	16.22±0.53 <sup>a</sup>	76.94±0.99 <sup>b</sup>	14.76±1.46 <sup>a</sup>	4.84±0.04 <sup>b</sup>
21	A	26.31±0.24 <sup>ab</sup>	16.22±0.45 <sup>a</sup>	76.63±0.42 <sup>a</sup>	14.58±1.00 <sup>a</sup>	4.59±0.07 <sup>a</sup>
	B	26.03±0.47 <sup>a</sup>	16.86±0.26 <sup>a</sup>	76.92±0.32 <sup>a</sup>	14.72±0.68 <sup>a</sup>	4.59±0.12 <sup>a</sup>
	C	25.26±0.54 <sup>a</sup>	15.70±0.73 <sup>a</sup>	77.72±0.44 <sup>a</sup>	15.17±0.96 <sup>a</sup>	4.82±0.10 <sup>b</sup>
35	A	26.57±0.18 <sup>a</sup>	16.73±0.14 <sup>a</sup>	76.36±0.26 <sup>a</sup>	14.43±0.89 <sup>a</sup>	4.60±0.08 <sup>a</sup>
	B	26.69±0.31 <sup>a</sup>	17.08±0.15 <sup>a</sup>	76.10±0.42 <sup>a</sup>	13.74±0.95 <sup>a</sup>	4.57±0.04 <sup>a</sup>
	C	24.98±0.87 <sup>b</sup>	14.85±0.87 <sup>b</sup>	78.01±1.13 <sup>b</sup>	15.38±1.65 <sup>a</sup>	4.78±0.03 <sup>b</sup>
56	A	26.11±0.26 <sup>a</sup>	16.08±0.19 <sup>a</sup>	76.97±0.27 <sup>a</sup>	15.32±0.15 <sup>a</sup>	4.52±0.03 <sup>a</sup>
	B	26.58±0.36 <sup>a</sup>	16.54±0.02 <sup>a</sup>	76.48±0.38 <sup>a</sup>	15.05±0.21 <sup>a</sup>	4.49±0.03 <sup>a</sup>
	C	25.11±0.79 <sup>b</sup>	15.70±0.13 <sup>b</sup>	77.87±0.59 <sup>a</sup>	15.25±0.69 <sup>a</sup>	4.81±0.02 <sup>b</sup>

\*A - commercial starter cultures; B - commercial starter cultures and adjunct autochthonous strain *Lb. paracasei* 08; C - whole autochthonous starter culture

\*\* Results are expressed as mean ± standard error of means; Means in each column and at the same age with the same letter did not differ significantly ( $P>0.05$ )

autochthonous LAB (cheese C) were characterized by significantly lower dry matter content ( $P < 0.05$ ) than cheeses A and B, the reason being probably the higher pH of these samples as a result of different acidogenic activities of LAB used, influencing the texture properties of cheeses (Awad, 2007).

Results of previous studies based on the use of commercial starter cultures showed no significant influence of adjunct cultures addition on the composition of different types of cheeses (Fenelon et al., 2002; Katsiari et al., 2002b; Michaelidou et al., 2003a). However, these results can be explained by the fact that adjunct cultures were generally not added for acid production in cheese but to enhance flavour.

The most of cheese composition parameters did not change significantly ( $P > 0.05$ ) during 8 weeks of ripening. However, a significant increase of dry matter content ( $P < 0.05$ ) after 7 days of ripening was probably due to the salt absorption in cheese and as a consequence, water diffusion from cheese into brine. It can be noticed that compositional parameters only slightly changed during 8 weeks of ripening. Generally, UF-Feta cheeses are characterised by close structure and a very low rate of syneresis during ripening (Karami et al., 2009).

pH values in the range of 4.85-4.52 remained during ripening at levels typical for white brined cheeses. Similar results were reported in the literature for UF-Feta cheeses (Karami et al., 2009). Cheese C was characterized by higher pH value compared to the other two cheeses. An explanation could be the lower acidogenic ability of autochthonous LAB compared to commercial starter cultures (Radulovic, 2007).

The pH values of cheeses decreased significantly ( $P < 0.05$ ) over the first 21 days and remained constant until the end of the ripening period. This can be related to the low lactose content (Miocinovic et al., 2011) and high buffering capacity of UF milk that significantly influence the acidification kinetics with LAB (Mistry, 2004).

## Proteolysis

### *Changes in nitrogen fractions*

Parameters of proteolysis (WSN/TN, PTAN/TN and PTAN/WSN) in low fat UF cheeses during ripening period are shown in Table 2.

The content of WSN/TN marked a constant increase during cheese ripening, especially after 7 and 21 days ( $P < 0.05$ ). The content of WSN/TN of cheeses was within the range of 11.12-11.42 % at the end of ripening, which is significantly lower compared to WSN/TN of traditionally made Feta cheese (Kandarakis et al., 2001). The contents of WSN/TN of brined cheeses made with conventional manufacturing process usually varies between 12 and 20 % (Abd El-Salam and Alichanidis, 2008). Different starter cultures showed no significant influence ( $P > 0.05$ ) on the rate of primary proteolysis determined by WSN/TN content. Similar results were presented by numerous authors who investigated the impact of different adjunct cultures on properties of various cheese types (Fenelon et al., 2002; Michaelidou et al., 2003a). Starter cultures were usually characterized by predominantly peptidase activity and did not show any significant influence on primary proteolysis (Sousa et al., 2001). However, some authors emphasized the significant influence of adjunct cultures on the rate of primary proteolysis, presumably as the result of proteolytic activity (Michaelidou et al., 2003a).

The content of PTAN/TN increased constantly during the entire ripening period. Statistically significant increase of PTAN/TN ( $P < 0.05$ ) was noticed after 7 and 21 days. It is important to note that cheeses made with autochthonous LAB (cheeses B and C) showed after 21 days of ripening a significantly higher content of PTAN/TN compared to control cheese A. Cheese C made with autochthonous LAB showed consistent trend of increase of the PTAN/TN content. This quite intensive rate of secondary proteolysis is presumably the result of specific proteolytic activity. The content of PTAN/TN was within the range of 1.76-2.04 % at the end of the ripening period of 56 days. It was significantly lower compared to PTAN/TN of traditionally made cheeses, which is often within 3-5 % (Abd El-Salam and Alichanidis, 2008). These results confirm the facts about slower proteolytic changes during the ripening of UF cheeses (Bech, 1993).

### *Polyacrylamide gel electrophoresis*

Urea and SDS electrophoretograms of low fat UF cheeses during ripening are shown in Figures 1 and 2. At all age, the PAGE patterns for the con-

Table 2. The proteolysis parameters of low fat UF cheeses during ripening

Days	Cheese*	WSN/TN (%)	PTAN/TN (%)	PTAN/WSN (%)
0	A	8.55±0.07 <sup>a</sup>	0.90±0.09 <sup>a</sup>	10.48±1.07 <sup>a</sup>
	B	8.62±0.31 <sup>a</sup>	0.88±0.14 <sup>a</sup>	10.18±1.57 <sup>a</sup>
	C	8.74±0.06 <sup>a</sup>	0.84±0.08 <sup>a</sup>	9.63±1.00 <sup>a</sup>
7	A	9.49±0.61 <sup>a</sup>	1.28±0.11 <sup>a</sup>	13.48±0.54 <sup>ab</sup>
	B	9.54±0.31 <sup>a</sup>	1.38±0.03 <sup>a</sup>	14.48±0.74 <sup>b</sup>
	C	9.77±0.57 <sup>a</sup>	1.22±0.23 <sup>a</sup>	12.45±1.76 <sup>a</sup>
21	A	10.35±0.27 <sup>a</sup>	1.41±0.04 <sup>a</sup>	13.61±0.68 <sup>a</sup>
	B	10.61±0.42 <sup>a</sup>	1.62±0.08 <sup>b</sup>	15.24±0.29 <sup>a</sup>
	C	11.02±0.70 <sup>a</sup>	1.70±0.07 <sup>b</sup>	15.46±1.43 <sup>a</sup>
35	A	10.46±0.10 <sup>a</sup>	1.38±0.06 <sup>a</sup>	13.21±0.66 <sup>a</sup>
	B	10.64±0.23 <sup>a</sup>	1.59±0.06 <sup>b</sup>	14.95±0.046 <sup>a</sup>
	C	11.64±0.42 <sup>b</sup>	1.73±0.12 <sup>b</sup>	14.89±1.57 <sup>a</sup>
56	A	11.12±0.26 <sup>a</sup>	1.76±0.12 <sup>a</sup>	15.84±0.75 <sup>a</sup>
	B	11.29±0.37 <sup>a</sup>	1.77±0.06 <sup>a</sup>	15.67±1.05 <sup>a</sup>
	C	11.42±0.13 <sup>a</sup>	2.04±0.14 <sup>b</sup>	17.90±1.32 <sup>b</sup>

\*A - commercial starter cultures; B - commercial starter cultures and adjunct autochthonous strain *Lb. paracasei* 08; C - whole autochthonous starter culture;

\*\*WSN/TN - water soluble nitrogen/total nitrogen matter, PTAN/TN - phosphotungstic soluble nitrogen/ total nitrogen matter, PTAN/WSN - phosphotungstic soluble nitrogen/ water soluble nitrogen

\*\*\*Results are expressed as mean ± standard error of means; Means of each parameter in the same column with the same letter do not differ significantly ( $P>0.05$ ).

trol and the experimental cheeses were similar, suggesting that the mode and rate of casein breakdown were similar in all cheeses. It is also evident that the rate of hydrolysis of the two caseins was different. The hydrolysis of  $\alpha_{s1}$ -casein was more intensive than that of  $\beta$ -casein. This indicates a more pronounced activity of residual chymosin than of plasmin.

According to the results of proteolysis parameters and casein degradation scheme, determined by electrophoresis, it can be concluded that proteolytic changes of low fat UF cheeses are weak as already shown by determination of the soluble nitrogen fractions in Table 2.

#### Microstructure

Microstructures of low fat UF cheeses after 8 weeks of ripening is shown in Figure 3.

The cheese mass appears to consist of dense and compact interlaced protein chains. The protein network is characterised by uniform aggregates of paracasein micelles. No significant difference between

cheeses made with different starter cultures are recognisable, indicating that the adjunct culture did not affect the microstructure examined by SEM. The average size of paracasein micelles was about 280 nm and pore size was about 3.4  $\mu\text{m}$  (data not show) and were not significantly different ( $P>0.05$ ). SEM micrographs of low fat UF cheeses were similar to the micrographs of feta cheese with reduced fat content (Sipahioglu et al., 1999).

#### Sensory analysis

The results of the sensory assessment of cheese quality during ripening are shown in Table 3. The appearance of all cheeses was considered very satisfactory at all sampling ages with no significant differences ( $P>0.05$ ) between cheeses as well as during the ripening period. However, significant differences ( $P<0.05$ ) in body and texture among cheeses made with commercial and autochthonous LAB were detected in all stages of ripening. Good textural quality of low fat UF cheese made with autochthonous LAB observed in the present study was probably due to its

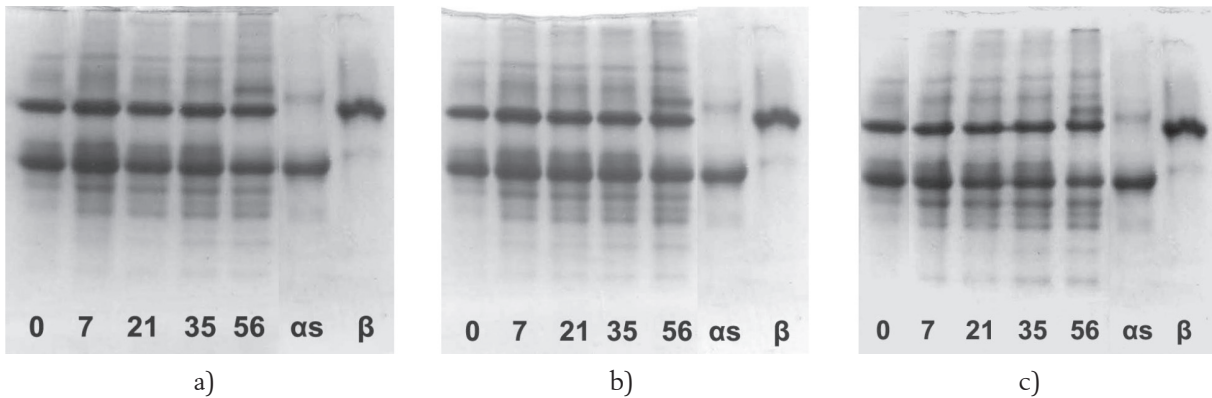


Figure 1. UREA electropherograms of low fat UF cheeses during 56 days of ripening: (a) control cheese with commercial starter culture; (b) cheese B with commercial starter culture and adjunct strain *Lb. paracasei* 08; (c) cheese C with autochthonous LAB.  $\alpha_s$  - Standard of  $\alpha_s$  casein;  $\beta$  - Standard of  $\beta$  casein

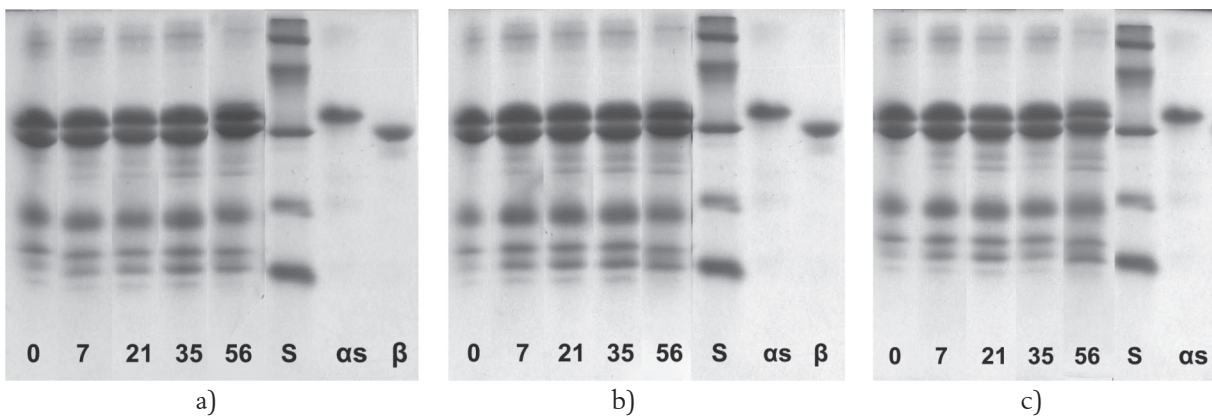


Figure 2. SDS PAGE electrophoretograms of low fat UF cheeses during 56 days of ripening: (a) control cheese with a commercial starter culture; (b) cheese B with a commercial starter culture and adjunct strain *Lb. paracasei* 08; (c) cheese C with autochthonous LAB. S - Molecular weights standard;  $\alpha_s$  - Standard of  $\alpha_s$  - casein;  $\beta$  - Standard of  $\beta$  casein

high moisture content as a consequence of a higher pH value. It is well-known that increasing the moisture level is a potential way to overcome textural disadvantages caused by fat reduction in cheese. The experimental low fat cheeses made with autochthonous LAB received significantly ( $P < 0.05$ ) higher flavour scores than the control cheese (Table 3). The results may be partly attributed to the slightly higher levels of soluble nitrogen compounds, but mainly to the significantly ( $P < 0.05$ ) higher concentrations of PTAN (small peptides and free amino acids). It is

noticeable that the addition of adjunct culture significantly improved the sensory quality of low fat cheese due to the specific peptidolytic enzymes that are presumably responsible for the formation of flavour compounds. The enhancement of flavour of various low-fat cheese varieties due to the addition of an adjunct culture is in agreement with the results of numerous research studies (Fenelon and Guinee, 2000; Katsiari et al., 2002a; Katsiari et al., 2002b).

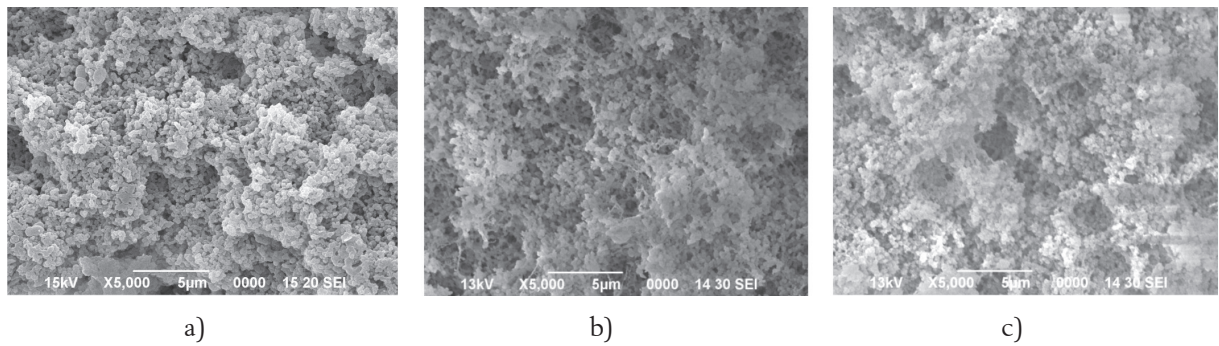


Figure 3. SEM micrographs of low fat UF cheeses; magnification 5000x; (a) control cheese with a commercial starter culture; (b) cheese B with a commercial starter culture and adjunct strain *Lb. paracasei* 08; (c) cheese C with autochthonous LAB

Table 3. Sensory properties of low fat UF cheeses during ripening

Days	Cheese	Exterior appearance	Interior appearance	Texture and consistency	Aroma	% Max. quality
7	A	4.97±0.05 <sup>a</sup>	4.92±0.14 <sup>a</sup>	3.71±0.18 <sup>a</sup>	3.50±0.10 <sup>a</sup>	78.17±1.04 <sup>a</sup>
	B	4.97±0.05 <sup>a</sup>	4.85±0.09 <sup>a</sup>	3.71±0.18 <sup>a</sup>	3.84±0.05 <sup>b</sup>	81.34±0.65 <sup>a</sup>
	C	4.93±0.12 <sup>a</sup>	4.88±0.11 <sup>a</sup>	4.19±0.11 <sup>b</sup>	4.44±0.10 <sup>c</sup>	89.86±1.56 <sup>b</sup>
21	A	4.97±0.05 <sup>a</sup>	4.97±0.05 <sup>a</sup>	3.78±0.17 <sup>a</sup>	3.49±0.15 <sup>a</sup>	78.62±2.53 <sup>a</sup>
	B	5.00±0.00 <sup>a</sup>	5.00±0.00 <sup>a</sup>	3.94±0.12 <sup>a</sup>	3.97±0.05 <sup>b</sup>	84.45±0.12 <sup>b</sup>
	C	5.00±0.00 <sup>a</sup>	5.00±0.00 <sup>a</sup>	4.56±0.10 <sup>b</sup>	4.29±0.08 <sup>c</sup>	90.72±0.66 <sup>c</sup>
35	A	4.89±0.02 <sup>a</sup>	4.92±0.14 <sup>a</sup>	3.50±0.00 <sup>a</sup>	3.46±0.07 <sup>a</sup>	76.66±0.63 <sup>a</sup>
	B	5.00±0.00 <sup>a</sup>	5.00±0.00 <sup>a</sup>	4.06±0.10 <sup>b</sup>	3.69±0.17 <sup>a</sup>	82.22±2.09 <sup>b</sup>
	C	5.00±0.07 <sup>a</sup>	5.00±0.02 <sup>a</sup>	4.56±0.19 <sup>c</sup>	4.29±0.14 <sup>b</sup>	90.72±2.36 <sup>c</sup>
56	A	4.89±0.07 <sup>a</sup>	4.92±0.13 <sup>a</sup>	3.50±0.13 <sup>a</sup>	3.46±0.19 <sup>a</sup>	76.66±2.03 <sup>a</sup>
	B	5.00±0.00 <sup>a</sup>	5.00±0.00 <sup>a</sup>	3.79±0.40 <sup>b</sup>	3.67±0.29 <sup>ab</sup>	80.62±4.89 <sup>a</sup>
	C	4.96±0.07 <sup>a</sup>	4.96±0.07 <sup>a</sup>	4.42±0.14 <sup>c</sup>	4.33±0.38 <sup>b</sup>	90.22±4.43 <sup>b</sup>

\*A - commercial starter cultures; B - commercial starter cultures and adjunct autochthonous strain *Lb. paracasei* 08; C - whole autochthonous starter culture;

\*\*Results are expressed as mean ± standard error of means; Means of each parameter in the same column with the same letter do not differ significantly ( $P>0.05$ )

## Conclusion

From the results of this study it can be concluded that autochthonous lactic acid bacteria strains show a great potential and can be used in cheese production, especially those with reduced fat content. The use of autochthonous lactic acid bacteria as an adjunct, or as a whole starter culture contributes to a higher rate of secondary proteolysis, which improves the sensory properties of low fat UF cheeses, pro-

duced according to the previously established production procedure (Miocinovic et al., 2011).

## Acknowledgements

This work was performed as part of Project No. 46009 funded by the Ministry of Education and Science, Republic of Serbia. We thank to laboratory technician T. Vukovic for technical assistance and help during cheese making.



*Utjecaj autohtonih bakterija mliječne kiseline na proteolizu, mikrostrukturu i senzorska svojstva niskomasnih UF sireva tijekom zrenja*

**Sažetak**

U radu je utvrđen utjecaj komercijalnih *Lactococcus lactis* ssp. *lactis* i *Lactococcus lactis* ssp. *cremoris* (sir A) i kombinacija autohtonih bakterija mliječne kiseline (BMK) *Lactobacillus paracasei* ssp. *paracasei* 08, *Lactococcus lactis* ssp. *cremoris* 656, *Lactococcus lactis* ssp. *lactis* 653 i (sir B i C) na sastav, proteolizu, mikrostrukturu i senzorska svojstva niskomasnih sireva tijekom zrenja. Niskomasni sirevi od ultrafiltriranog mlijeka (UF) proizvedeni su prema definiranom tehnološkom postupku proizvodnje miješanjem UF proteina mlijeka u prahu, obranog mlijeka i vrhnja. Nije utvrđen značajan utjecaj različitih BMK na sastav, primarnu proteolizu i mikrostrukturu. Sirevi sa autohtonim BMK odlikovali su se višim stupnjem sekundarne proteolize, kao i boljim senzorskim svojstvima u odnosu na kontrolni sir.

*Ključne riječi:* sir, starter kultura, mikrostruktura, senzorska svojstva

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